AMENDED CLAIMS
[received by the International Bureau on 06 January 2005 (06.01.05);
original claims 1-22 replaced by new claims 1-21 (4 pages)]

1. A process for the preparation of cross linked enzyme crystals of hydrolases, and oxidoreductases which are solvent tolerant, thermostable and shear resistant, the process comprising the steps of:

(a) crystallizing the enzymes in aqueous buffer with a suitable salts and cosolvents in the presence of surfactants at a temperature ranging between 4^0 to 10^0 C for a period ranging between 5 hr. to 15 days to obtain the crystals of the protein having a particle size ranging between 50 to 150 microns;

(b) reacting the crystals of the enzyme obtained in step (a) with a multifunctional crosslinking agent in the presence of buffer of pH ranging between 3-8 at a temperature ranging between 4^0 to 25^0 C to get the crossed linked enzyme crystal;

(c) washing the cross linked crystals with a reagent that is capable of removing the excess of the said multifunctional cross linking reagent so as to obtain the washed cross linked protein; and

(d) coating cross linked protein crystals with a suitable surfactant, and lyophilizing it to obtain the stable product.

2 The process as claimed in claim 1, wherein said enzymes selected from the group consisting of hydrolases and the said enzyme is a starch hydrolyzing amylase namely glucoamylase.

3. A process as claimed in claim 1, wherein said oxidoreductase enzyme is a plant peroxidase.

4. The process as claimed in claims 1 to 3 wherein said oxidase is selected from the group of plant peroxidases consisting of Horse radish, Ipomea or Saccharum peroxidases.

5. A process as clamed in claim 1 wherein the crystallizing salt is sulphate of ammonium or sodium either as saturated solution or crystals.

6. A process as claimed in claim 1 wherein the said buffer used for the cross linked glucoamylase preparation is an aqueous buffer of 10mM -0.5M of acetate having a pH of 4.5.

7. A process as claimed in claim 1 wherein the said buffer used for the cross linked peroxidase preparation is an aqueous buffer of 10mM -0.5M phosphate or tris having pH of 6.5-8.0.

8. A process for the preparation of the cross linked protein enzyme crystal as claimed in claim 1, wherein the said co-solvent is an alcohol having a concentration of 1-20% , example 2-methyl,2,4 pentane diol; 2-propanol; 1,5 pentane diol, ethanol, methanol, isoamyl alcohol.

9. A process as claimed in claims 1 to 8, wherein said crystal is a microcrystal of 150 microns or less.

10. A process as claimed in claim 1, wherein the cross linking reagents used is glutaraldehyde, and starch dialdehyde.

11. A process as claimed in claim 1, wherein the said surfactant used is anionic, non-ionic, or cationic.

12. A process as claimed in claims 1 to 11 wherein the cationic surfactant used is cetyl trimethyl ammonium bromide or cetrimide.

13. A process as claimed in claims 1-12 wherein the anionic surfactant used is dioctyl sulfosuccinate Aerosol OT.

14. A process as claimed in claims 1 to 13 wherein the non-ionic surfactant used is selected from the group consisting of alkyl phenol ethoxylate, sorbitan trioleate, sorbitan tristerate . Examples Tween 20, Tween 80 and Triton X-100.

15. A process as claimed in claims 1 to 14 wherein the said surfactant provides a weight ratio of crosslinked enzyme crystals to surfactant between about 1:1,and about 1:5, preferably between about 1:1 and about 1:2 and is in a lyophilized form.

16. The process as claimed in claim 1, wherein the cross linked gulcoamylase is active in 1:1 mixture of water organic solvents n-dodecane; n-hexane; chloroform; and dimethyl sulphoxide.

17. A process as claimed in any of the preceding claims, wherein the said crosslinked enzyme crystal is having resistance to exogenous proteolysis, such that said crosslinked enzyme crystal retains at least 91% of its initial activity after incubation for three hours in the presence of a concentration of Protease that causes the soluble uncrosslinked form of the enzyme that is crystallized to form said enzyme crystal that is crosslinked to lose at least 94% of its initial activity under the same conditions, wherein said crystal is in lyophilized form.

18. The process as claimed in claim 1, wherein the cross linked Peroxidases are active in organic solvents like toluene; 80% dioxane, chloroform; 2-propanol; chloroform; acetone; ethanol; acetonitrile; methnol; and dioxane.

19. A process of continuous generation of glucose solution making use of the cross linked enzyme crystal as claimed in claims 1 to 18, wherein the said cross linked glucoamylase crystals are packed in a jacketed column for the continuous saccharification of starch solution having a concentration of 1-20% preferably 4-10%(W/V) at pH 4.5 and at 60° C with a yield of 110g glucose /L/hour at a residence time of 7.6 min.

20. A process of continuous generation of glucose solution making the cross linked glucoamylase crystal as claimed in claim 19, wherein the said enzyme can also act upon a solution of 1-30%(W/V)of maltodextrin of DE 10-15 preferably 10%(W/V) maltodextrin with a DE of 10-14 at a pH of 4.5, at 60° C thereby producing glucose solution within 1-8 min with a yield of 463 to 714 g/L/h.

21. A process as claimed in claims 1 to 18 wherein the crystals of plant peroxidase especially Horse radish peroxidase produces 2, 4 dimethyl phenol dimmer from monomer dissolved either in 2-propanol or toluene and the catalysis carried out at 50°C for 30 min. in the presence of 30% H_2O_2.

STATEMENT UNDER ARTICLE 19(1)

The subject application relates to a process for the preparation of cross linked enzyme crystals and the use of the product made by the process.

All the 22 claims have been replaced by the new amended claims from 1 to 21. However, the claims 1 to 18 have been suitably amended and claims 19 to 21 have been added. The claiming features have already been disclosed in the complete specification. The claims have been amended to make them more definitive and explicit.